The Concept of Entropy and its Concavity for a Finite Protein in its Environment: An exact study on a Square Lattice

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Abstract

We consider a general lattice model of a finite protein in its environment and calculate its Boltzmann entropy $S(E)$ as a function of its energy E in a microcanonical ensemble, and Gibbs entropy $\overline{S}(\overline{E})$ as a function of its average energy $\overline{E}$ in a canonical ensemble by exact enumeration on a square lattice. We find that because of the finite size of the protein, (i) the two are very different and $\overline{S}(\overline{E}) > S(\overline{E})$, (ii) $S(E)$ need not be concave while $\overline{S}(\overline{E})$ is, and (iii) $\overline{S}(\overline{E})$ is relevant for experiments but not $S(E)$, even though $S(E)$ is conceptually more useful. We discuss the consequences of these differences. The results are general and applicable to all finite systems.

Self-assembling small proteins are a prime example of small systems, and can fold into their native states (of minimum free energy) without any chaperones. They have been extensively investigated recently using lattice models by thermodynamic principles [1]. The smallest known natural protein is Trp-Cage derived from the saliva of Gila monsters and has only 20 residues. Their first-principle study requires short ranged model energetics that, while remaining independent of the thermodynamic state of the protein such as its conformation, temperature T, pressure P, etc., determine the native state(s), and has to be judiciously chosen to give a unique and right native state. It should be stressed that proteins in Nature are never isolated but always occur in an environment such as a cell controlled by the temperature T. Thus, the proper way to study proteins is to consider the canonical ensemble (CE), and not the microcanonical ensemble (ME). Moreover, the two ensembles are most probably not *equivalent* for a finite system. Despite this, investigations using ME are very common for proteins. Therefore, their predictions must be carefully examined and compared with those from CE, keeping in mind their possible non-equivalence. Unfortunately, this does not seem to be practiced in the field, which as we will establish here may be quite dangerous for finite systems such as proteins.

The ME entropy is given by the Boltzmann relation $S(E) \equiv \ln W(E)$, where $W(E)$ is the number of protein conformations of energy E. Since folding is a conformational change into the native state, the conformational entropy $S(E)$ is believed to play a central role in determining the way folding occurs into compact native states along a very large number of microscopic pathways that connect them to myriad unfolded conformations. It also characterizes the potential energy landscape [2, 3, 4]. It is a well-established tenant of *macroscopic* thermodynamics that $W(E)$ decreases with falling energy E as folding proceeds ($\partial S/\partial E \geq 0$); consequently, the energy landscape is expected to possess a structure that narrows down with falling energy, such as a funnel [5]. It is known that the entire thermodynamics is contained in $S(E)$, which must be *concave* [6] for a macroscopic system. This concavity is built-in in the random energy model [7], which has been extensively employed for proteins; see [4] for example, which also shows that the energy gap above the ground state (lowest energy state) is crucial for foldability. The resulting lack of concavity has been used as a sign of a first-order folding transition for small proteins by several workers. It should be noted that there are other idealized physical models such as the KDP model that freeze into the ground state at finite T due to a similar gridlock [8, 9].

A proper model of a protein should satisfy certain principles [10], one of which is the requirement of cooperativity. The sequence of residues also plays an important role in determining the native state [11]. However, there is no consensus for general energetics to describe all proteins, and there remains a certain amount of freedom in the choice, at least in modeling. It is widely recognized that secondary structures are also important in the folding process [12]. The simplest model is the standard model, which classifies the 20 different residues into two, H (hydrophobic) and P (hydrophilic), and allows only nearest-neighbor attractive HH interaction $e_{\rm HH}$ (set $= -1$ in some predetermined unit) to provide good hydrophobic cores [12]; however, consideration of local energetics of the 20 kinds of residues [13] is also common. It is found that the introduction of multibody interactions enhances cooperativity [14], and should not be neglected. It is important, therefore, to investigate the energetics effects on the form of $S(E)$, which to the best of our knowledge has not been studied carefully.

The direct experimental approaches (primarily, X-ray crystallography or NMR spectroscopy) to determine energetics requires information about the *typical* conformation associated with the average energy $\overline{E}(T)$. Thus, CE must be used to determine the dependence of the *canonical* entropy $S(T)$ on $\overline{E}$, given by the Gibbsian relation $S(T) = -\sum p(\Gamma,T)\ln p(\Gamma,T)$, where $p(\Gamma,T)$ is the probability to be in the conformation Γ at T. For a macroscopic system, $S(\overline{E})$ and $S(T)$ are the same so that $S(T)$ allows us to identify conformations of average energy $\overline{E}$. Their equality is crucial for the direct experimental approaches in which conformations associated with $\overline{E}$ need to be identified as *typical*. Thus, it is also important to verify if the two entropies are the same for finite proteins that are of interest here. If not true, the interpretation of experi-

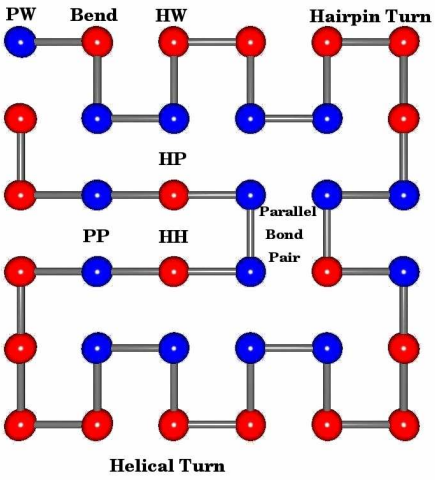

FIG. 1: A 2-d model of a finite protein on a square lattice. The red spheres represent hydrophobic sites and the blue spheres represent hydrophilic sites.

mental data for the energetics could be *incorrect*. This will become a limitation of the direct experimental techniques.

Model The interplay of intra-protein molecular interactions, the interaction with the surrounding, and the residue sequence to give rise to the folded native state is quite intricate and far from a basic understanding at present; much remains to be understood. It has been argued that conflicts among interactions also play a significant role in folding [15]. In general, the model should contain various interactions relevant not only for protein folding and various secondary substructures like helix formation in the native state, but also for proteins considered as semi-flexible heteropolymers [16] with certain specific sequences [17]. The model should also contain solvation effect, as all protein activity occurs in the presence of water or solvent. In this work, we use such a model, which has been investigated by us recently [18] in different limits one of which is the standard model described above. Here, we only report some unexpected results for finite proteins, which have not been noted earlier to the best of our knowledge. It should, however, be recognized that finite proteins cannot undergo a sharp folding transition. This issue is not relevant as we are only interested in comparing $S(E)$ and $\overline{S}(\overline{E})$.

We consider a protein with M residues in a given sequence on a square lattice, with one of its ends fixed at the origin so that the total number of conformations W for a finite protein remains finite even on an infinite lattice. We generalize a recent model [16], in which the number of bends $N_{\rm b}$, pairs of parallel bonds $N_{\rm p}$, and hairpin turns $N_{\rm hp}$ characterize the semiflexibility; see Fig. 1, where we show a protein in its compact form so that all the solvent molecules (W) such as water are expelled from the inside and surround the protein. We do not allow any free volume. The red spheres denote hydrophobic residues (H) and blue spheres denote hydrophilic (i.e., polar) residues (P). The nearest-neighbor distinct pairs PP, HH, HP, PW and HW between the residues and the water are also shown, but not the contact WW. Only three out of these six contacts are independent on the lattice [19], which we take to be HH, HW, and HP pairs. A bend is where the protein deviates from its collinear path. Each hairpin turn requires two consecutive bends in the same (clockwise or counterclockwise) direction; see Fig.1. Two parallel bonds one lattice spacing apart form a pair (p). We also consider the number of helical turns $N_{\rm hl}$. On a square lattice, a "helical turn" is interpreted as two consecutive hairpin turns in opposite directions; see Fig.1. The corresponding energies are $e_{\rm b}$, $e_{\rm P}$, $e_{\rm hp}$, and $e_{\rm hl}$, respectively. The pair interaction energies are $e_{\rm HH} = -1$, $e_{\rm HW}$, and $e_{\rm HP}$, and the pair numbers are $N_{\rm HH}$, $N_{\rm HW}$, and $N_{\rm HP}$, corresponding to the HH, HW, and HP, respectively. respectively. We let $\mathbf{e}$ denote the entire ordered set $\{e_i\}$,with i ordered as b,p,hp,hl,HH,HW, and HP, and $\mathbf{e}'$ the ordered set $\{e_i\}$ excluding $e_{\rm HH}(=-1)$. Similarly, $\mathbf{N} \equiv \mathbf{N}(\Gamma) \equiv \{N_i(\Gamma)\}$, and $\mathbf{N}'$ denotes all $\{N_i\}$ but $N_{\rm HH}$. The three most often energy choices we have made are: (A) $\mathbf{e}' = 0$, (B) $\mathbf{e}' = (a, -a, -2a, -a, 25a, 5a)$, $a = 1/50(<< 1)$, (C) $\mathbf{e}' = (\, b, -b, -b, -b, 2b, b), b = 1/3(\simeq 1)$. The standard model is (A). In the model (B), we have most other interactions much weaker than $|e_{\rm HH}|$, while they are comparable to $|e_{\rm HH}|$ in the model (C). Thus, (B) is closer to (A) than (C) is. Despite this, we will see that (B) and (C) behave very different from (A). It should be noted that W does not depend on the model; it is its partition into $W(E)$ that depends on the model. Thus, the shape of the energy landscape changes from model to model, but not its total "area" which is given by W [5]. We have also considered random, ordered and fixed sequences. We consider *compact* and *unconstrained* protein conformations [18] separately. We have found that in the majority of cases that we have investigated, the sequence containing a repetition of PPHH gives rise to the lowest energy or very close to it.

The energy of a given conformation Γ is

$$E(\Gamma) \equiv \mathbf{e} \cdot \mathbf{N}(\Gamma) \equiv \sum e_i N_i(\Gamma). \qquad (1)$$

We partition W according to $\mathbf{N}$ or E, so that $W \equiv \sum_{\mathbf{N}} W(\mathbf{N}) \equiv \sum_E W(E)$, where $W(\mathbf{N})$ [or $W(E)$] is the number of conformations for a given set $\mathbf{N}$ [or E]. On a lattice, E remains a discrete variable, but this fact is not important for our final conclusions as we will discuss below. In the standard model, $E = -N_{\rm HH}$. It is clear from

$$W(N_{\rm HH}) \equiv \sum W(N_{\rm HH}, \mathbf{N}'), \qquad (2)$$

that the entropy $S(N_{\rm HH}) = \ln W(N_{\rm HH})$ for a given $N_{\rm HH}$, regardless of $\mathbf{N}'$, is maximum in the standard model [20] and provides a possible justification of the observation made in [14]. A protein with a given $N_{\rm HH}$ will probe many more states in the standard model, where there is no energetic penalty to explore all possible $\mathbf{N}'$, than in any other

model with energetic penalty, which then slows down it approach to the native state. Thus, it is important to hav non-zero $\mathbf{e}'$ to step up the approach to the native state. (I is highly likely that the native states in different models ar different, but this does not affect the above conclusion. There is another important consequence of $\mathbf{e}' = 0$. Th fluctuations in the corresponding N_i are maximum as ther is no penalty no matter what $\mathbf{N}'$ is. The protein will spen a lot of time probing a large number of conformations cor responding to the maximum fluctuations in $\mathbf{N}'$. This als suggests that we need to go beyond the standard model t describe proteins that fold fast.

The canonical probability distribution for Γ i $p(\Gamma, T) \equiv e^{-\beta E(\Gamma)}/Z(T)$, where

$$Z(T) \equiv \sum_{\Gamma} e^{-\beta E(\Gamma)} \equiv \sum_{E} W(E) e^{-\beta E}, \qquad (3$$

the partition function, describes the finite protein thermo dynamics; here, $\beta \equiv 1/T$ (we set the Boltzmann constan $k_{\rm B} = 1$). The distribution $p(\Gamma)$ can be used to define th average $<>$ of any thermodynamic quantity (also denote by an overbar in the following) such as $\overline{\mathbf{N}}(T) \equiv < \mathbf{N} >$ and $\overline{E}(T) \equiv < E > \equiv \mathbf{e} \cdot \overline{\mathbf{N}}(T)$ [$\overline{e} \equiv \overline{E}/M$]. The fre energy $F(T) \equiv -T \ln Z(T)$ gives the canonical entrop $S(T) \equiv -\partial F(T)/\partial T$, which satisfies the conventional thermodynamic relation $F(T) \equiv \overline{E}(T) - TS(T)$, and the Gibbsian relation quoted above, as can be easily checked. Both $S(T)$ and $\overline{E}(T)$ are *continuous function* (except possibly at a phase transition, which is not relevant here as we are dealing with a finite protein) of the continuous variable T. Moreover, $F(T)$ is monotonically decreasing with T as expected.

Since the derivative $\partial\overline{E}/\partial T$ is non-negative as can be easily checked, $\overline{E}$ can be *inverted* to express T as a function $T(\overline{e})$, which then allows us to express $S(T)$ as an explicit function $\overline{S}(\overline{E}) \equiv S[T(\overline{e})]$ of $\overline{E}$. The entropy $\overline{S}(\overline{E})$ can be thought of as the *canonical equivalence* of the microcanonical entropy $S(E)$. However, they are two *different* quantities for finite proteins. In the first place, $S(E)$ is a discrete function since E is discrete, while $\overline{S}(\overline{E})$ is a continuous function since $\overline{E}$ is continuous. In the second place, $\overline{S}(\overline{E}) \geq S(\overline{E})$, the equality holding as $M \to \infty$ [5]. To demonstrate this, let us assume that $E = \overline{E}$ is one of the energies in the sum in (3). We then rewrite $\overline{S}(\overline{E}) = \ln Z + \overline{E}/T$, and evaluate $\overline{W}(\overline{E}) \equiv \exp[\overline{S}(\overline{E})]$:

$$\overline{W}(\overline{E}) = W(\overline{E}) + \sum_{E \neq \overline{E}} W(E) e^{-\beta(E-\overline{E})}; \qquad (4)$$

hence, $\exp[\overline{S}(\overline{E})] \geq \exp[W(\overline{E})]$ as asserted above. The difference between them is due to the non-negative last term in (4), which vanishes as $N \to \infty$. In case, $\overline{E}$ is not one of the energies in the sum, we can use a suitable interpolation to define $\overline{W}(\overline{E})$, without affecting the conclusion [18]. The above proof does not depend on the discrete nature of the energies in ME; thus, it is also valid for continuum models. We show in Fig.2 the exactly enumerated

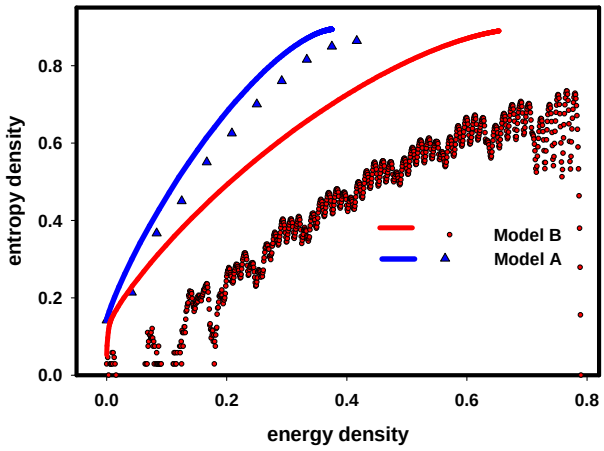

FIG. 2: Continuous $\overline{s}(\overline{e})$ (blue and red curves), and discrete $s(e)$ (blue and red points) for a given sequence ($M = 24$, unrestricted);. The bands in s become more pronounced and their separations decrease as M increases. Note a clear band in s at low energies and the native state, disjoint from the rest of the bands.

entropies per residue $s(e) \equiv (1/M)S(E)$ (red curve as a guide through discrete points) and $\overline{s}(\overline{e}) \equiv (1/M)\overline{S}(\overline{E})$ (blue curve) for the model (B) ($M = 24$; unrestricted conformations) as a function of the discrete variable $e \equiv E/M$ or $\overline{e}$. In addition, we also see a distinct *band structure* in $s(e)$ that gives rise to regions of non-concavity [6], which is related to the nature of the interactions and has no implication for any phase transition as we will discuss below.

It is easily seen that the canonical entropy function satisfies the conventional thermodynamic relation [5]

$$\partial\overline{S}(\overline{E})/\partial\overline{E} = 1/T, \qquad (5)$$

and is, therefore, concave ($\partial^2\overline{S}(\overline{E})/\partial\overline{E}^2 < 0$) [6]. On the other hand, the microcanonical entropy need *not* be concave; see Fig.2, where the bands seen in $s(e)$ have both positive and negative slopes, which is in contradiction with (5) valid for $\overline{s}(\overline{e})$. The non-concave $S(E)$ does not violate finite system thermodynamics. The canonical entropy is the physical entropy for proteins in its environment and remains concave in Fig. 2 as required by thermodynamic stability.

To understand the absence of concavity, we first consider the model (A). In all cases that we have studied [18], $S(E) = S(N_{\rm HH})$ is found to be concave. The number of states $W(N_{\rm HH})$ can be partitioned into $W(N_{\rm HH}, \mathbf{N}')$; see (2). In the model (B), $\mathbf{e}' \simeq 0$; therefore, most of the conformations in $W(N_{\rm HH})$ have energies that are close to $-N_{\rm HH}$; some of them will have energies that are outside the range $(-N_{\rm HH}-1, -N_{\rm HH}+1)$. The resulting $S(E)$ associated with this $N_{\rm HH}$ is almost concave, as seen in each band in Fig.2. This then gives rise to the lack of concavity in the region where two nearby bands overlap. The number of bands equals the number of possible values of $N_{\rm HH}$

in the model (A). These convex portions of $s(e)$ disappear and $s(e)$ approach $\overline{s}(\overline{e})$ from below as $M \to \infty$ [5]. But for finite systems, the convex regions persists. The band structure persists for all sequences that we have checked. The additional energies in the model (C) provide enough spread for bands to overlap; this reduces the size of convex regions. Even here, we have found that the band nature survives at the upper and the lower ends of the energy. Thus, we are confident that convex regions in $S(E)$ will exist in any realistic model of a protein. Their presence, however, does not imply any phase transition, as $\overline{S}(\overline{E})$ is always concave. This is true even though we note from Fig.2, that there is a clear gap at the lowest energy. The energy gap causes convexity in $S(E)$, but not in $\overline{S}(\overline{E})$.

Because of conformational changes during folding, the folding is believed to be governed by the multiplicity $W(E)$, which in turn governs the energy landscape for which $W(E)$ represents the "surface area" of the hypersurface of the landscape at energy E [5]: each point on the hypersurface represents a conformation. The lack of concavity discovered here has a profound effect on the *shape* of the landscape. It no longer narrows down as E decreases. It will be interesting to pursue the consequences of this shape modification. This is beyond the scope of the present work, but we hope to consider it elsewhere. It is evident, and as discussed above, several different $\mathbf{N}$ will usually mix together for a given E, except in the model (A) in which $E = -N_{\rm HH}$ so that $W(E) = W(N_{\rm HH})$. There will be a certain landscape topology for the standard model, which will change with $\mathbf{e}'$. From (2), it is evident that the landscape will become drastically narrower for $\mathbf{e}' \neq 0$. The total "surface area" W of the landscape does not change with $\mathbf{e}'$, even though the allowed energies change and they become closer. The landscape narrowing and closeness of energies at constant W make the approach to native state presumably $\overline{E}$more directional and fast.

Since it is CE that is relevant for a real protein in its environment, it is the canonical multiplicity $\overline{W}(\overline{E})$ that is relevant for folding. As shown above, it continuously increases with $\overline{E}$, until we reach at infinite temperatures. Thus, the narrowing of the landscape with non-zero $\mathbf{e}'$ may not be as relevant for protein folding as the observation that $\overline{W}(\overline{E}) > W(\overline{E})$. From (4), we observe that $\overline{W}(\overline{E})$ gets contribution from *all* conformations, not just the conformations in W $(\overline{E})$. Thus, it may be misleading to think that a finite protein at a given T only probes some typical conformations of average energy $\overline{E}$. (It is possible that $\overline{E}$ may not even be an allowed energy E.) It also probes native state, though its probability is going to be small. As T is reduced, this probability increases. In addition, the protein restricts its search to effectively a smaller set of conformations, closer in energy. It would be interesting to follow the consequence(s) of this observation.

In conclusion, we observe that the microcanonical entropy, which dictates the form of energy landscape, does not satisfy concavity; however, this violation does not imply any impending phase transition; the latter requires investigating the behavior of the canonical entropy, which always satisfies concavity. However, nearly all works on protein thermodynamics have not paid any attention to this issue. This may be dangerous. The most surprising result is the tremendous difference between the two entropies: the canonical entropy is almost twice as big as the microcanonical entropy at intermediate energies, but much larger at low energies. Its implication for experimental data interpretation, as noted above, needs to be further pursued.

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